Novel Highly Oxygenated Bisabolane Sesquiterpenes from *Cremanthodium discoideum*

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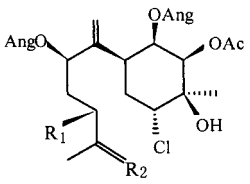
Five new highly oxygenated bisabolane sesquiterpenes (**1**−**5**) were isolated from *Cremanthodium discoideum*. Their structures were elucidated on the basis of spectroscopic analysis and chemical transformations. The structure and relative stereochemistry of **1** were determined by single-crystal X-ray crystallography on the acetate derivative, **1a**. Compound **1** showed antibacterial activity against *Bacillus acidilatici* and *Bacillus subtilis.*

The genus *Cremanthodium* (Compositae) is widespread in the mountains of the Himalayas and contiguous climatic regions. Several plants of this genus have been used as Tibetan traditional herbal medicine to treat fever, inflammation, pain, and apoplexy.[1,2] Our group has investigated *C. ellisii*, and a number of highly oxygenated bisabolane sesquiterpenes were characterized.[3] Bisabolane compounds possess such interesting biological properties as antitumor, antibacterial, and insect antifeedant activities.[4−7] We have now studied *C. discoideum* Maxim. and describe herein the structure elucidation of five new bisabolane sesquiterpenes (**1**−**5**). The structure and relative stereochemistry of the acetate (**1a**) of **1** were established unambiguously by single-crystal X-ray crystallography. Compound **1** exhibited antibacterial activity against *Bacillus acidilatici* and *Bacillus subtilis*.



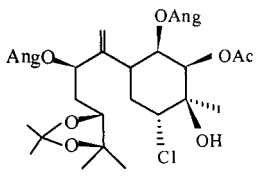
1: R_1=OH, R_2=Me/OCH_3

1a: R_1=OAc, R_2=Me/OCH_3

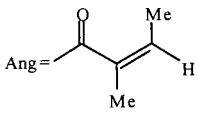
2: R_1=OH, R_2=Me/OH

3: R_1=OH, R_2=CH_2

4: R_1=Cl, R_2=Me/OH



5



Results and Discussion

Compound **1** was obtained as colorless gum. Its FABMS gave quasi-molecular ion peaks at *m/z* 559 $[M + H]^+$, 565 $[M + Li]^+$, and 581 $[M + Na]^+$, accompanied by isotopic peaks at *m/z* 561, 567, and 583, respectively, with the ratio between the two being 3:1, suggesting the presence of a chlorine atom. The molecular formula of **1** was determined as $C_{28}H_{43}O_9Cl$ by HRFABMS. The IR spectrum showed absorption bands for hydroxyl groups (3562, 3519 cm^{-1}, br), ester carbonyl groups (1746, 1718 cm^{-1}), a double bond (1648 cm^{-1}), and a carbon−chlorine band (753 cm^{-1}). The ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively) showed that there were an acetoxy, two angeloyloxy groups, two hydroxyl groups, and a methoxy group in **1**. This finding was also supported by the characteristic ion fragments at *m/z* 543 $[M - CH_3]^+$, 485 $[M - C(OCH_3)Me_2]^+$, 458 $[M - AngOH]^+$, 358 $[M - 2AngOH]^+$, 73 $[C(OCH_3)Me_2]^+$, and 43 $[CH_3CO]^+$ in its EIMS. The ^{13}C NMR spectrum of **1** showed signals for 28 carbons, and DEPT spectrum showed the presence of nine methyls, three methylenes, eight methines, and eight quaternary carbons. In consideration of seven unsaturations and the above-mentioned groups, compound **1** was proposed to be a bisabolane sesquiterpene with a C_{15} skeleton (three methyls, three methylenes, six methines, and three quaternary carbons) having a terminal double bond (δ_H 5.28, 4.99, 1H each, s; δ_C 115.0 C, 146.0 CH_2).

The $^1H-^1H$ COSY and HMQC NMR spectra of **1** showed the presence of three moieties, −CH(2)−CH(1)−CH(6)−, −CH(4)−CH_2(5)−CH(6)−, and −CH(8)−CH_2(9)−CH(10)−, which were connected by the following long-range $^1H-^{13}C$ correlations: C-2/H-4, H-15; C-3/H-2, H-4, H-15; C-6/H-1, H-14, H-14′; C-7/H-6, H-8, H-14, H-14′; C-8/H-9, H-14, H-14′; C-11/H-10, H-12, H-13. The ^1H NMR spectrum of **1** gave proton signals for four oxygen-bearing methines and a chloro-bearing methine (δ_H 5.63, 5.23, 4.21, 5.59, and 3.46). The ^{13}C NMR spectrum also showed the corresponding carbon signals (δ_C 70.5, 70.8, 64.4, 73.9, and 72.9) and two oxygen-bearing quaternary carbons (δ 74.2, 76.9). In the HMBC experiment, the quaternary carbons (δ 165.5 and 167.3) of two angeloyloxy groups resulted in cross-peaks with the signals at δ 5.63 (H-1) and δ 5.59 (H-8), which indicated that angeloyloxy groups were attached to C-1 and C-8, respectively. The quaternary carbon (δ 169.7) of the acetoxy group showed a cross-peak with the H-2 proton (δ 5.23), indicating that an acetoxy group was attached to C-2. Similarly, the protons (δ 3.22) of the methoxy group showed a cross-peak with C-11. The two hydroxyl groups in the molecule were tertiary and secondary, respectively. By comparison, the chemical shift of C-3 (δ 74.2) of **1** with that of a novel compound (δ 74.3) in the literature,[3] it was suggested that the tertiary hydroxyl group was affixed to C-3. In the ^{13}C NMR spectrum of **1**, the signal of C-4 was shifted to higher field (δ 64.4) compared with that of C-10 (δ 72.9), which suggested that the chlorine atom was at C-4 and the secondary hydroxyl group at C-10. Acetylation of **1** afforded **1a**. In the ^1H NMR spectrum of **1a**, the proton signal of H-4 was unaffected, which meant that the chlorine atom was located at the C-4 position in accordance with this being an acetylation-resistant functional group. The H-10 ^1H NMR signal of **1a** showed a downfield shift (from δ 3.46 to 5.09), and the hydroxyl proton signal (δ 7.23) at C-10 was missing in the ^1H NMR spectrum, which also gave evidence for the hydroxyl at C-10 in **1**.

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**, **1a**, **2**, **3**, **4**, and **5** (400 MHz, $CDCl_3$) [a]

proton	1	1a	2	3	4	5
1	5.63 (t, 3.2)	5.63 (t, 2.9)	5.63 (t, 3.2)	5.63 (t, 3.2)	5.62 (t, 3.2)	5.63 (t, 3.2)
2	5.23 (d, 3.2)	5.23 (d, 2.9)	5.23 (d, 3.2)	5.23 (d, 3.2)	5.21 (d, 3.2)	5.23 (d, 3.2)
4	4.21 (dd, 2.9, 2.8)	4.21 (dd, 3.0, 2.7)	4.21 (dd, 2.8, 2.6)	4.21 (dd, 2.8, 2.6)	4.22 (dd, 2.7, 2.5)	4.22 (dd, 2.9, 2.7)
5β	2.64 (ddd, 15.5, 12.6, 2.9)	2.63 (ddd, 15.6, 12.4, 3.0)	2.65 (ddd, 15.3, 12.4, 2.8)	2.66 (ddd, 15.3, 12.7, 2.6)	2.74 (ddd, 15.5, 12.8, 2.7)	2.64 (ddd, 15.3, 12.6, 2.7)
5α	1.89 (m)	1.89 (m)	1.89 (m)	1.89 (m)	1.74 (ddd, 15.5, 2.7, 2.6)	1.89 (m)
6	3.20 (br dd, 12.6, 2.3)	3.17 (br d, 12.4)	3.22 (br d, 12.4)	3.20 (br d, 12.7)	3.17 (br d, 12.8)	3.21 (br d, 12.6)
8	5.59 (dd, 10.4, 2.9)	5.22 (dd, 10.5, 3.0)	5.57 (dd, 10.5, 2.4)	5.59 (dd, 10.2, 2.9)	5.68 (dd, 10.1., 4.6)	5.48 (dd, 8.0, 5.5)
9	1.98 (m)	2.08 (m)	2.12 (m)	1.74 (m)	2.24 (m)	1.80 (m)
9′	1.61 (m)	1.81 (m)	1.65 (m)	1.57 (m)	2.09 (m)	1.80 (m)
10	3.46 (br d, 10.5)	5.09 (dd, 10.8, 2.1)	3.53 (br d, 10.5)	4.00 (dd, 9.6, 2.2)	3.57 (dd, 11.5, 1.7)	3.69 (dd, 9.0, 2.4)
12	1.14 (s)	1.16 (s)	1.58 (s)	5.00 (s)	1.32 (s)	1.25 (s)
12′				4.99 (s)		
13	1.14 (s)	1.14 (s)	1.57 (s)	1.76 (s)	1.31 (s)	1.10 (s)
14	5.28 (s)	5.26 (s)	5.29 (s)	5.27 (s)	5.42 (s)	5.29 (s)
14′	4.99 (s)	4.98 (s)	5.01 (s)	4.85 (s)	5.15 (s)	5.00 (s)
15	1.34 (s)	1.34 (s)	1.34 (s)	1.34 (s)	1.34 (s)	1.34 (s)
17						1.42 (s)
18						1.27 (s)
OCH_3−11	3.22 (s)	3.24 (s)				
OAc−2	2.06 (s)	2.07 (s)	2.06 (s)	2.06 (s)	2.06 (s)	2.06 (s)
OAc−10		2.10 (s)				
OH−3	3.56 (s)	3.54 (s)		3.55 (s)		
OH−10	2.73 (br s)		2.82 (br s)			
OAng−1	6.10 (qq, 7.3, 1.4)	6.08 (qq, 7.3, 1.4)	6.11 (qq, 7.3, 1.5)	6.10 (qq, 7.3, 1.5)	6.10 (qq, 7.4, 1.6)	6.07 (qq, 7.6, 1.4)
	1.94 (dq, 7.3, 1.4)	1.94 (dq, 7.3, 1.4)	1.95 (dq, 7.3, 1.4)	1.95 (dq, 7.3, 1.3)	1.94 (dq, 7.4, 1.5)	1.93 (dq, 7.6, 1.5)
	1.89 (dq, 1.4, 1.4)	1.89 (dq, 1.4, 1.4)	1.89 (dq, 1.5, 1.4)	1.89 (dq, 1.5, 1.3)	1.90 (dq, 1.6, 1.5)	1.89 (dq, 1.4, 1.5)
OAng−8	6.10 (qq, 7.3, 1.4)	6.10 (qq, 7.3, 1.4)	6.13 (qq, 7.3, 1.5)	6.13 (qq, 7.3, 1.5)	6.14 (qq, 7.4, 1.6)	6.10 (qq, 7.6, 1.4)
	2.00 (dq, 7.3,1.5)	2.00 (dq, 7.3, 1.5)	2.00 (dq, 7.3,1.4)	2.00 (dq, 7.3,1.4)	2.00 (dq, 7.4, 1.6)	2.00 (dq, 7.6, 1.4)
	1.93 (dq, 1.4, 1.5)	1.93 (dq, 1.4, 1.5)	1.93 (dq, 1.4,1.5)	1.95 (dq, 1.5, 1.4)	1.95 (dq, 1.6, 1.6)	1.94 (dq, 1.4, 1.4)

[a] Multiplicity and coupling constant(s) in Hz are in parentheses, chemical shifts are shown in δ values (ppm) with TMS as internal standard.

Table 2. ^{13}C NMR Spectral Data of Compounds **1**, **1a**, **2**, **3**, **4**, and **5** (100.6 MHz, $CDCl_3$) [a]

carbon	1	1a	2	3	4	5
1	70.5 (d)	70.3 (d)	70.2 (d)	70.5 (d)	70.6 (d)	70.6 (d)
2	70.8 (d)	70.8 (d)	70.7 (d)	70.7 (d)	70.8 (d)	70.8 (d)
3	74.2 (s)	74.1 (s)	73.8 (s)	74.2 (s)	74.1 (s)	74.2 (s)
4	64.4 (d)	64.2 (d)	64.2 (d)	64.3 (d)	64.5 (d)	64.4 (d)
5	29.7 (t)	29.6 (t)	29.5 (t)	29.8 (t)	30.4 (t)	29.6 (t)
6	35.1 (d)	35.4 (d)	35.5 (d)	35.1 (d)	33.6 (d)	34.8 (d)
7	146.0 (s)	146.0 (s)	146.0 (s)	146.6 (s)	142.4 (s)	145.3 (s)
8	73.9 (d)	73.3 (d)	73.2 (d)	73.5 (d)	76.3 (d)	74.8 (d)
9	35.9 (t)	33.3 (t)	36.5 (t)	40.0 (t)	35.5 (t)	33.4 (t)
10	72.9 (d)	72.2 (d)	74.9 (d)	71.5 (d)	69.0 (d)	79.8 (d)
11	76.9 (s)	75.4 (s)	74.0 (s)	145.7 (s)	72.4 (s)	79.9 (s)
12	19.7 (q)	21.0 (q)	28.3 (q)	111.0 (t)	26.1 (q)	25.6 (q)
13	19.7 (q)	22.1 (q)	28.1 (q)	18.1 (q)	25.6 (q)	22.8 (q)
14	115.0 (t)	114.9 (t)	115.0 (t)	115.4 (t)	119.1 (t)	115.9 (t)
15	23.7 (q)	23.7 (q)	23.7 (q)	23.8 (q)	23.8 (q)	23.7 (q)
16						106.9 (s)
17						28.4 (q)
18						26.7 (q)
OCH_3−11	49.2 (q)	49.7 (q)				
OAc−2	169.7 (s)	169.7 (s)	169.6 (s)	169.7 (s)	169.6 (s)	169.7 (s)
	20.6 (q)	20.6 (q)	20.5 (q)	20.6 (q)	20.6 (q)	20.6 (q)
OAc−10		170.4 (s)				
		20.6 (q)				
OAng−1	165.5 (s)	165.4 (s)	165.3 (s)	165.6 (s)	165.5 (s)	165.4 (s)
	126.6 (s)	126.5 (s)	126.4 (s)	126.5 (s)	126.5 (s)	126.6 (s)
	138.7 (d)	138.5 (d)	138.9 (d)	139.3 (d)	139.5 (d)	138.4 (d)
	15.6 (q)	15.6 (q)	15.6 (q)	15.7 (q)	15.7 (q)	15.6 (q)
	20.5 (q)	20.4 (q)	20.4 (q)	20.5 (q)	20.5 (q)	20.4 (q)
OAng −8	167.3 (s)	166.7 (s)	167.3 (s)	167.7 (s)	166.4 (s)	166.7 (s)
	127.6 (s)	127.6 (s)	127.4 (s)	127.4 (s)	127.6 (s)	128.0 (s)
	139.7 (d)	139.7 (d)	139.9 (d)	139.9 (d)	139.5 (d)	139.5 (d)
	15.8 (q)	15.6 (q)	15.7 (q)	15.8 (q)	15.9 (q)	15.7 (q)
	20.6 (q)	20.4 (q)	20.5 (q)	20.5 (q)	20.8 (q)	20.5 (q)

[a] Multiplicity is deduced by DEPT, chemical shifts are shown in δ values (ppm).

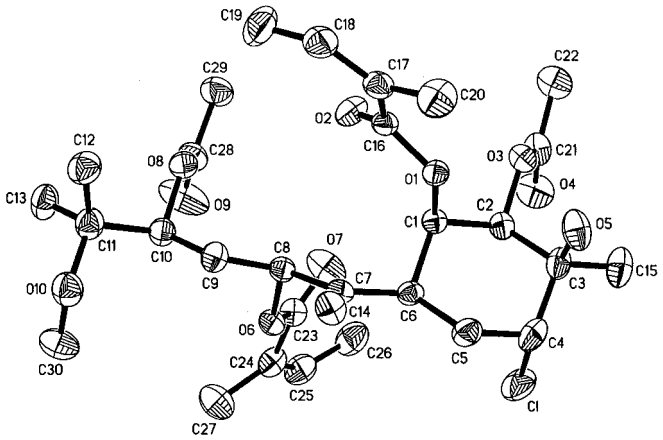

Figure 1. ORTEP drawing of **1a** at 50% probability level

The relative stereochemistry of **1** was studied by a H^1−H^1 NOESY experiment. Strong NOE correlations were observed between (a) H-1 and H-2, (b) H-1 and H-6, (c) H-2 and H-6, and (d) H-2 and H-15. It was suggested that these protons were α-oriented, which was in agreement with the small coupling constants ($J_{1,2} = 3.2$, $J_{1,6} = 2.3$ Hz) observed. In the ^1H NMR spectrum, H-4 showed a one-proton triplet with $J = 2.8$ Hz, due to two protons (H-5β and H-5α) at C-5; so, therefore, H-4 was β-oriented. Thus, the structure of **1** was determined as 2β-acetoxy-4α-chloro-1β,8-diangeloyloxy-3β,10-dihydroxy-11-methoxybisabol-7(14)-ene. To confirm the structure of **1**, compound **1a** was prepared, and the relative stereochemistry was assigned by single-crystal X-ray crystallographic analysis (Figure 1).

Compound **2** was obtained as a colorless gum. Its FABMS gave a quasi-molecular ion peak at *m/z* 545 $[M + H]^+$, accompanied by an isotopic peak at *m/z* 547, with the ratio between being 3:1. Its molecular formula $C_{27}H_{41}O_9Cl$ was determined by HRFABMS and was 14 mass units less than that of **1**. In the EIMS, characteristic fragments at *m/z* 385 $[M - CMe_2OH - AngOH]^+$ and 285 $[M - CMe_2OH - 2AngOH]^+$ were visible. The NMR and IR data showed a close resemblance to those of **1**, but methoxy group signals were absent in the ^1H and ^{13}C NMR spectra of **2**. In the ^{13}C NMR spectrum, the ^{13}C NMR signal of C-11 was shifted upfield from δ 76.9 to 74.0, and the signals of C-12 and C-13 were shifted downfield from δ 19.7 to 28.1 and 28.3, respectively. Accordingly, compound **2** is the demethyl analogue of **1** and was determined as 2β-acetoxy-4α-chloro-1β,8-diangeloyloxy-3β,10,11-trihydroxybisabol-7(14)-ene.

Compound **3** was obtained as a colorless gum. Its FABMS gave quasi-molecular ion peaks at *m/z* 533 $[M + Li]^+$ and 549 $[M + Na]^+$, accompanied by isotopic peaks at *m/z* 535 and 551, respectively. The intensity ratio of the isotopic peaks for each group was nearly 3:1. The HR-FABMS of **3** showed a $[M + H]^+$at *m/z* 527.2416 (calcd 527.2412), with a molecular formula of $C_{27}H_{39}O_8Cl$. The ^1H and ^{13}C NMR spectra of **3** were very similar to those of **1**, with the exception of the absence of a methoxyl at C-11 and a methyl group at C-12. Instead, a terminal double bond was apparent between C-11 and C-12, which was confirmed by the signals of C-11 (δ 145.7) and C-12 (δ 111.0) in the ^{13}C NMR spectrum, and the olefinic proton signals at δ 5.00 (12-H, s) and 4.99 (12′-H, s) in the ^1H NMR spectrum. The methyl proton signal at C-13 of **3** was shifted downfield to δ 1.76, because the methyl was in the deshielding region of a double-bond group. Compound **3** was therefore assigned as 2β-acetoxy-4α-chloro-1β,8-diangeloyloxy-3β,10-dihydroxybisabol-7(14),11(12)-diene.

Compound **4** was obtained as colorless gum. Its FABMS gave quasi-molecular ion peaks at *m/z* 569 $[M + Li]^+$ and

Table 3. Antibacterial Activity of Compound **1**[a]

	B. acidilatici	*B. aeruginosus*	*B. subtilis*
compound **1**	+	−	++
chloramphenicol	++	++	++

[a] Antimicrobial activity is defined as follows: ++ = the diameter is equal to 13−15 mm; + = equal to 10−12 mm; and − = less than 9 mm.

585 $[M + Na]^+$, accompanied by isotopic peaks at *m/z* 571 and 573, 587 and 589, respectively (their relative abundance ratios were approximately 9:6:1). Therefore, it could be suggested that there were two chlorine atoms in **4**. Its molecular formula was determined as $C_{27}H_{40}O_8Cl_2$ by HRFABMS. A comparison of ^{13}C NMR spectral data of **4** with those of **2** revealed that they both are similar, except that the signals in **4** showed upfield shifts from δ 74.9 to 69.0 for C-10 and from 74.5 to 72.4 for C-11, due to the replacement of a chlorine group. The EIMS fragment peaks at *m/z* 355 $[M - C(OH)Me_2CHCl - AngOH]^+$, 303 $[M - C(OH)Me_2 - 2AngOH]^+$, 255 $[M - C(OH)Me_2CHCl - 2AngOH]^+$, provided useful information on connectivity, allowing replacement of a hydroxyl group at C-10 by a chlorine atom. Compound **4** was determined as 2β-acetoxy-1β,8-diangeloyloxy-4α,10-dichloro-3β,11-dihydroxybisabol-7(14)-ene.

Compound **5** was obtained as colorless gum. The ^{13}C NMR and DEPT spectral data showed that compound **5** contained 10 methyl, three methylene, eight methine, and nine quaternary carbons, representing an additional $C(CH_3)_2$ unit when compared to **2**. The FABMS of compound **5** gave quasi-molecular ion peaks at *m/z* 591 $[M + Li]^+$ and 607 $[M + Na]^+$, accompanied by isotopic peaks at *m/z* 593 and 609, respectively, with the ratio between the two being 3:1. Its molecular formula was determined as $C_{30}H_{45}O_9Cl$ by HRFABMS. The EIMS gave characteristic ion fragments at *m/z* 569 $[M - CH_3]^+$, 484 $[M - AngOH]^+$, 384 $[M - 2AngOH]^+$, and 129 $[C(CH_3)_2CHO_2C(CH_3)_2]^+$, which also supported the presence of a $C(CH_3)_2$ group. The ^1H and ^{13}C NMR spectral data of **5** were almost identical to those of **2** except in the region of C-10 and C-11, indicating that the $C(CH_3)_2$ group was attached to two oxygen atoms connected at C-10 and C-11. Therefore, compound **5** was assigned as 2β-acetoxy-4α-chloro-1β,8-diangeloyloxy-3β-hydroxy-10,11-isopropoxybisabol-7(14)-ene.

Previously, it has been reported that chlorine-bearing sesquiterpenes occur in the genus *Centaurea* (Compositae).[8] In our isolation process, our extract did not come into contact with hydrochloric acid, and we did not use chloroform as a solvent of elution. Therefore, we feel convinced that compounds **1**−**5** are actual natural products. Compound **1** exhibited antibacterial activity against *B. acidilatici* and *B. subtilis*. The results were compared with chloramphenicol and are summarized in Table 3.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler micromelting point apparatus and are uncorrected. Optical rotations were measured using a Perkin−Elmer 241 polarimeter in $CHCl_3$. IR spectra using KBr disks were recorded on a Nicolet 170-SX spectrometer. ^1H NMR, ^{13}C NMR, and 2D NMR spectra were measured on a Bruker AM 400-FT−NMR spectrometer. EIMS and FABMS were recorded on a VG-ZAB−HS mass spectrometer, HRFABMS measurements were recorded on a Finnigan-4510 mass spectrometer. Single-crystal X-ray analysis was performed on a Siemens P4 diffractometer with graphite-monochromated Mo Kα radiation and an ω-2θ scan. Column chromatography was carried out on Si gel (200−300 mesh), and TLC and preparative TLC were performed on Si gel GF_{254}.

Plant Material. *Cremanthodium discoideum* was collected in Qinghai Province of the People's Republic of China in August 1994, and identified by Prof. Zexian Peng of the Department of Biology, Lanzhou University. A voucher specimen (no. 9481) has been deposited in the Herbarium of the Department of Chemistry, Lanzhou University, People's Republic of China.

Extraction and Isolation. Air-dried whole plants of *C. discoideum* (6 kg) were powdered and extracted three times (each for a week) with petroleum ether−Et_2O−MeOH (1:1:1) at room temperature, and the solvent was removed under reduced pressure to give a residue (270 g). This extract was subjected to column chromatography on Si gel with a gradient of petroleum ether−Me_2CO (50:1−1:1) to afford seven crude fractions (A−G). Fractions A, B, F, and G contained triterpenoids, fat, steroids, and steroidal glycosides and were not further investigated. Fractions C and D, of similar composition, were pooled (30 g, petroleum ether−Me_2CO, 10:1−6:1). This was then separated by column chromatography over Si gel with a petroleum ether−Me_2CO gradient (10:1−4:1) to give six fractions. Fractions 3−5 (petroleum ether−Me_2CO 8:1−6:1) were combined and further separated by column chromatography on Si gel with C_6H_6−Me_2CO (6:1) and produced two fractions. The first of these was subjected to preparative TLC with C_6H_6−Me_2CO (8:1) as developing solvent (three developments) and further purified by preparative TLC with petroleum ether−Et_2O (1:1.5, two developments), affording 40 mg of **2** and 15 mg of **5**, respectively. The second fraction was also subjected to preparative TLC with petroleum ether−Et_2O (1:2, two developments), then purified by preparative TLC with C_6H_6−Me_2CO (6:1, two developments) to afford 80 mg of **1**, 19 mg of **3**, and 10 mg of **4**, respectively.

2β-Acetoxy-4α-chloro-1β, 8-diangeloyloxy-3β,10-dihydroxy-11-methoxybisabol-7(14)-ene (1): colorless gum; $[\alpha]^{20}_D$ −53.3° (*c* 1.0, $CHCl_3$); IR ν_{max} 3562, 3519 (OH), 1746, 1230 (OAc), 1718, 1648 (C=CCO_2R), 846 (C=C), 753 (C−Cl) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 543 $[M - CH_3]^+$ (0.3), 485 $[M - C(OCH_3)Me_2]^+$ (0.4), 458 $[M - AngOH]^+$ (0.3), 427 $[M - OCH_3 - AngOH]^+$ (1), 385 $[M - AngOH - C(OCH_3)Me_2]^+$ (4), 358 $[M^+ - 2AngOH]^+$ (1), 285 $[M^+ - 2AngOH - C(OCH_3)Me_2]^+$ (2), 83 $[C_4H_7CO]^+$ (58), 73 $[C(OCH_3)Me_2]^+$ (100), 55 $[83 - CO]^+$ (27), 43 $[CH_3CO]^+$ (19); FABMS *m/z* 559 $[M + H]^+$, 565 $[M + Li]^+$, 581 $[M + Na]^+$; HRFABMS *m/z* 559.272975 $[M + H]^+$ (calcd for $C_{28}H_{44}O_9Cl$, 559.267386).

4α-Chloro-2β,10-diacetoxy-1β,8-diangeloyloxy-11-methoxy-3β-hydroxybisabol-7(14)-ene (1a): Treatment of 20 mg of compound **1** overnight with Ac_2O in pyridine (1:1) followed by preparative TLC gave 15 mg of **1a**: colorless crystals; mp 86−89 °C; $[\alpha]^{20}_D$ −60° (*c* 1.0, $CHCl_3$); IR ν_{max} 3561 (OH), 1744, 1233 (OAc), 1719, 1649 (C=CCO_2R), 846 (C=C), 752 (C−Cl) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m/z* 569 $[M - OCH_3]^+$ (0.04), 508 $[M - CH_3OH - AcOH]^+$ (0.4), 500 $[M - AngOH]^+$ (0.3), 408 $[M - CH_3OH - AcOH - AngOH]^+$ (1), 308 $[M - CHOH_3 - AcOH - 2AngOH]^+$ (0.3), 249 $[M - OCH_3 - 2AcOH - 2AngOH]^+$ (1), 83 $[C_4H_7CO]^+$ (56), 73 $[C(OCH_3)Me_2]^+$ (100), 59 $[AcO]^+$ (1), 55 $[83 - CO]^+$ (15), 43 $[CH_3CO]^+$ (10).

X-ray Crystallography of 1a.[9] Colorless prismatic crystals of **1a** were established as having the monoclinic space group $P2_1$, crystal data: $C_{30}H_{45}O_{10}Cl$, molecular wt 601.11, a = 10.556 (1), b = 13.723 (2), c = 11.809 (1) Å, β = 109.71(1)°, V = 1610.4 (3) Å^3, Z = 2 and with a calculated density of 1.240 g/cm^3, Mo Kα (λ = 0.71073 Å), μ = 0.171 mm^{-1}, $F(000)$ = 644. Intensity data were measured up to 54 of 2θ (h = 0 to 13, k = 0 to 17, l = −15 to 14). The number of reflections measured was 3992 (total) and 3600 (unique). The structure was solved by direct method (SHELXS-86 and SHELXS-93 software package) and was refined by the full-matrix least-squares method with observed 2283 [I > 2σ(I)] reflections. Nonhydrogen atoms were refined with anisotropic displacement param-

eters, and hydrogen atoms in calculated positions were included but not refined. The final *R* factor is 0.039.

2*β*-Acetoxy-4α-chloro-1*β*,8-diangeloyloxy-3*β*,10,11-trihydroxybisabol-7(14)-ene (2): colorless gum; $[\alpha]^{20}_D$ −62.4° (*c* 2.0, $CHCl_3$); IR ν_{max} 3564, 3499 (OH), 1733, 1230 (OAc), 1720, 1647 (C═CCO_2R), 846 (C═C), 734 (C−Cl) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m*/*z* 545 $[M + H]^+$ (0.06), 529 $[M - CH_3]^+$ (0.6), 526 $[M - H_2O]^+$ (0.1), 444 $[M - AngOH]^+$ (0.1), 426 $[M - H_2O - AngOH]^+$ (2), 408 $[M - HCl - AngOH]^+$ (0.2), 385 $[M - CMe_2OH - AngOH]^+$ (2), 344 $[M - 2AngOH]^+$ (0.03), 285 $[M - CMe_2OH - AngOH]^+$ (0.3), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (46), 43 $[CH_3CO]^+$ (37); FABMS *m*/*z* 545 $[M + H]^+$; HRFABMS *m*/*z* 545.243441 $[M + H]^+$ (calcd for $C_{27}H_{42}O_9Cl$, 545.251736).

2*β*-Acetoxy-4α-chloro-1*β*,8-diangeloyloxy-3*β*,10-dihydroxybisabol-7(14), 11(12)-diene (3): colorless gum; $[\alpha]^{20}_D$ −55.2° (*c* 0.6, $CHCl_3$); IR ν_{max} 3565, 3510 (OH), 1746, 1231 (OAc), 1718, 1648 (C═CCO_2R), 846 (C═C), 734 (C−Cl) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m*/*z* 526 $[M]^+$ (0.04), 508 $[M - 18]^+$ (0.3), 491 $[M - Cl]^+$ (0.2), 426 $[M - AngOH]^+$ (1), 390 $[M - HCl - AngOH]^+$ (0.1), 326 $[M - 2AngOH]^+$ (0.6), 290 $[M - HCl - 2AngOH]^+$ (0.1), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (37), 43 $[CH_3CO]^+$ (25); FABMS *m*/*z* 533 $[M + Li]^+$, 549 $[M + Na]^+$; HRFABMS *m*/*z* 527.241585 $[M + H]^+$ (calcd for $C_{27}H_{40}O_8Cl$, 527.241171).

2*β*-Acetoxy-1*β*,8-diangeloyloxy-4α,10-dichloro-3*β*,11-dihydroxybisabol-7(14)-ene (4): colorless gum; $[\alpha]^{20}_D$ −67.8° (*c* 0.05, $CHCl_3$); IR ν_{max} 3561, 3510 (OH), 1744, 1231 (OAc), 1719, 1646 (C═CCO_2R), 846 (C═C), 734 (C−Cl) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m*/*z* 562 $[M]^+$ (0.1), 527 $[M - Cl]^+$ (0.1), 467 (0.39), 465 (2), 463(3), 462 $[M - AngOH]^+$ (2), 427 $[M^+ - Cl - AngOH]^+$ (5), 404 $[M - C(OH)Me_2 - AngO]^+$ (0.3), 362 $[M^+ - 2AngOH]^+$ (0.4), 355 $[M - C(OH)Me_2CH\ Cl - AngOH]^+$ (0.1), 304 $[M - C(OH)Me_2 - 2AngO]^+$ (2), 303 $[M - C(OH)Me_2 - 2AngOH]^+$ (2), 255 $[M - C(OH)Me_2CHCl - 2AngOH]^+$ (0.4), 292 $[M^+ - 2Cl - 2AngOH]^+$ (0.1), 83 $[C_4H_7CO]^+$ (100), 59 $[M - C(OH)Me_2$ or $AcO]^+$ (15), 55 $[83 - CO]^+$ (34), 43 $[CH_3CO]^+$ (21); FABMS *m*/*z* 567 $[M + Li]^+$, 585 $[M + Na]^+$; HRFABMS *m*/*z* 563.225090 $[M + H]^+$ (calcd for $C_{27}H_{41}O_8Cl_2$, 563.217849).

2*β*-Acetoxy-4α-chloro-1*β*,8-diangeloyloxy-3*β*-hydroxy-10,11-isopropoxybisabol-7(14)-ene (5): colorless gum; $[\alpha]^{20}_D$ −53.3° (*c* 1.0, $CHCl_3$); IR ν_{max} 3576 (OH), 1748, 1231 (OAc), 1719, 1650 (C═CCO_2R), 844 (C═C), 743 (C−Cl) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; EIMS *m*/*z* 569 $[M - CH_3]^+$ (5), 484 $[M - AngOH]^+$ (1), 384 $[M^+ - 2AngOH]^+$ (1), 129 $[C(CH_3)_2CHO_2C(CH_3)_2]^+$ (18), 83 $[C_4H_7CO]^+$ (100), 55 $[83 - CO]^+$ (37), 43 $[CH_3CO]^+$ (35); FABMS *m*/*z* 591 $[M + Li]^+$, 607 $[M + Na]^+$; HRFABMS *m*/*z* 585.282951 $[M + H]^+$ (calcd for $C_{30}H_{46}O_9Cl$, 585.283067).

Antimicrobial Assays. Three strains of bacteria, *B. acidilatici* (MCCB 44102), *B. aeruginosus* (MCCB 10104), and *B. subtilis* (MCCB 26501), were cultured under agar. The paper-disk method [10] was used as an antimicrobial test, 0.1 mL of 100 *μ*g/mL of compound **1** or chloramphenicol (used as positive control) was added to each piece of paper. After 1 h, the disks were dried and placed onto a culture dish at 37 °C for 24 h. The antimicrobial activity was calculated by the diameter (in millimeters) of the antibacterial circle. Each test was performed in duplicate.

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(9) Crystallographic data for **1a** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-(0)1223-336033 or E-mail: deposit@ccdc.cam.ac.uk).
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